THE MECHANISM OF THE ABDERHALDEN REACTION WITH BACTERIAL SUBSTRATES *

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Since the appearance of Abderhalden's dialysis reaction and his explanation of the mechanism as a digestion of substrate by serum ferments, various phenomena have been observed which throw some doubt on the validity of this theory. The fact that a positive reaction, as evidenced by an increase in dialyzable, ninhydrin-reacting substances, may result from the combination of inorganic materials with serum, indicates that his explanation does not cover all conditions. It is obvious that the positive reaction in this case cannot be the result of the digestion of substrate. It may be assumed rather that the reaction is primarily an adsorption process, the secondary stage of which is the autodigestion of the serum protein by ferments inherent in the serum itself. In fact, proof of this hypothesis has been offered by many observers, among whom are De Waele,[1] Plaut,[2] Bronfenbrenner[3] and Jobling, Eggstein, and Petersen.[4]

While arriving at the same conclusion in regard to the fact that the Abderhalden reaction is essentially dependent upon an adsorption process, these investigators hold different views in regard to individual factors influencing the reaction. De Waele[1] considers that the mechanism of the reaction consists in a change in the state of solubility of the serum globulins. This change may be due to the physical action of non-specific inorganic substances, or it may be produced in a specific manner by the interaction of a serum and its homologous substrate. He places emphasis upon the fact that the reaction, under certain conditions, may be specific. Specificity, however, should be ascribed, not to a multiplicity of individual ferments, but to factors which modify the state of solubility of the globulins.

Plaut[2] also suggests that organic substrates may induce through physical processes the formation of substances which will react positively to ninhydrin.

Jobling, Eggstein, and Peterson[4] are convinced that no actual digestion of substrate can take place. In support of this view they have demonstrated that placental tissue, when subjected to treatment with pregnant serum, presents a definite increase in nitrogen-containing material. This increase is due to an adsorption of antitrypsin, as is indicated by the fact that placenta which has remained in contact with pregnant serum, is subsequently more resistant to tryptic digestion than normal placenta. Thus any digestion which may occur

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[1] Ztschr. f. Immunitätsf. O., 1914, 22, p. 170.
[2] München. med. Wchnschr., 1914, 61, p. 238.
[3] Jour. Exper. Med., 1915, 21, p. 221.
[4] Ibid., p. 239.

is due, not to the activity of specific ferments upon their substrates, but to the serum proteases, which, following the adsorption of the inhibitory agents (antiferments), are free to act upon the serum proteins.

Bronfenbrenner[3] agrees with the view of the last-mentioned investigators that the reaction is a phenomenon of adsorption. With inorganic substrates it depends simply upon physical adsorption of serum antiferments, as by kaolin, barium sulfate, or substances of a similar nature. With organized substrates a more complicated mechanism is involved. This, Bronfenbrenner has resolved into 2 distinct phases. The first comprises a sensitization of the substrate by the specific elements of the immune serum with the resultant adsorption of antitrypsin. The second is the autodigestion of the serum.

Employing placental tissue and pregnant serum, Bronfenbrenner found that the reaction was specific, but that the specificity was limited entirely to the first phase of the reaction. This he demonstrated by allowing a serum and substrate to remain in contact in the cold, no digestion occurring. When, however, serum and substrate were separated and the serum was allowed to dialyze alone at incubator temperature, a positive reaction was obtained in those cases in which the serum was specific. That a change in the serum had occurred was evident. That a change had also taken place in the substrate was shown through the fact that thenceforth this sensitized substrate induced the appearance of dialyzable products in any serum. In the specificity of substrate sensitization he sees a phenomenon analogous to an antigen-antibody reaction — an observation which had already been made by De Waele.[1]

As Bronfenbrenner's work had been confined entirely to tissue substrates, it was considered of interest to determine whether the same principles were operative in the case of bacterial substrates, and whether the limits of specificity were as clearly defined.

Accordingly, the experiments here to be reported were conducted in strict conformity with the technic of Bronfenbrenner. Immune sera were obtained from rabbits which had received repeated injections of typhoid bacilli ("Rawling's"), paratyphoid A bacilli, and Staphylococcus aureus. Duplicate animals were immunized with each strain. The bacterial substrates were prepared as in work previously reported by Smith.[5] The dialysis thimbles were Schleicher and Schüll's No. 579A, and had been tested for both permeability and impermeability.

The rabbits were bled from 5 to 7 days after the last injection and the serum of each rabbit was combined with its homologous substrate and also with the two non-specific substrates. For each serum there was a control tube with no substrate. Controls were also made upon substrates alone. The serum-substrate contacts, as well as the controls, were held in the cold for 16 hours. The tubes were then centrifugated and during centrifugation they were kept packed in ice that there might be no possibility of a digestion of the serum during the interval required for its complete separation from the substrate. After centrifugation the clear serum was removed from the substrate and held in the ice-box until it was transferred to dialyzing sacs. Dialysis was allowed to proceed at 37 C. for 16 hours, after which the usual ninhydrin test was made. The substrates which had been collected by centrifugation were washed twice in normal salt solution to remove all traces of serum. Each of the substrates was then divided into 4 portions, to 3 of which fresh serum was added

[5] Jour. Infect. Dis., 1915, 16, p. 319.

from the three types of immunized rabbits, and to the fourth portion fresh serum of a normal rabbit. These were again allowed to remain in contact in the cold for 16 hours. The tubes were then centrifugated cold; the serum was removed, placed in dialyzing sacs, and left at incubator temperature for 16 hours. The dialysates were tested for the presence of substances reacting positively to ninhydrin.

The serum of each of the immune animals, as well as that of 2 normal rabbits, was subjected to this treatment. The procedure applied to Rabbit 16, immunized to typhoid, represents a typical test. For clearness the procedure is presented in the following tables and is divided into the two phases.

TABLE 1

PROCEDURE I—FIRST PHASE: SERUM AND SUBSTRATE MIXED AND PLACED ON ICE FOR 16 HOURS; THEN THE MIXTURES WERE CENTRIFUGATED IN THE COLD, AND THE SERUM DIALYZED FOR 16 HOURS AT 37 C.

	July 7		July 9	
Tube	Serum (1 c.c. + Substrate (2 c.c.)		Ninhydrin Test	Sac
a	16	Typhoid	+	a
a	16	Typhoid	+	a
b	16	Paratyphoid A	—	b
b	16	Paratyphoid A	—	b
c	16	Staphylococcus aureus	—	c
c	16	Staphylococcus aureus	—	c
d (control)	16	None	—	d
e (control)	None	Typhoid	—	e
f (control)	None	Paratyphoid A	—	f
g (control)	None	Staphylococcus aureus	—	g

TABLE 2

PROCEDURE II—SECOND PHASE: THE TWO TUBES OF EACH TYPE OF SUBSTRATE WERE UNITED, WASHED TWICE WITH SALT SOLUTION, DIVIDED INTO 4 PORTIONS, AND COMBINED WITH FRESH SERUM FROM RABBITS 16 (TYPHOID), 18 (PARATYPHOID A), 20 (STAPHYLOCOCCUS AUREUS), AND 22 (NORMAL). THESE MIXTURES WERE THEN PLACED ON ICE 16 HOURS; THEN CENTRIFUGATED IN THE COLD, AND THE SERUM DIALYZED 16 HOURS AT 37 C.

	July 8		July 10	
Tube	Serum (1 c.c. + Substrate (1 c.c.)		Ninhydrin Test	Sac
1	16	Typhoid a*	+	1
2	16	Paratyphoid A b	—	2
3	16	Staphylococcus aureus c	—	3
4	16	None	—	4
5	18	Typhoid a	+	5
6	18	Paratyphoid A b	+	6
7	18	Staphylococcus aureus c	—	7
8	18	None	—	8
9	20	Typhoid a	+	9
10	20	Paratyphoid A b	—	10
11	20	Staphylococcus aureus c	+	11
12	20	None	—	12
13	22	Typhoid a	+	13
14	22	Paratyphoid A b	—	14
15	22	Staphylococcus aureus c	—	15
16	22	None	—	16

* The letter following the name of the substrate indicates the tube in Procedure I from which the substrate was taken. For example, Typhoid "a" represents a typhoid substrate which has already been in contact with a typhoid serum.

It is evident from the work outlined in Procedure I that an immune serum, when combined in the cold with its specific substrate and subsequently dialyzed alone, undergoes some degradation, as is indicated by the positively reacting dialysates in Sacs "a." This same serum, when combined in an identical manner with heterologous substrates, as in Tubes "b" and "c," is not digested, nor is there any digestion in Control Tubes "d" and "e." Obviously, during the contact in the cold the specific substrate enters into some reaction with the serum. That this reaction is dependent upon specific factors is indicated by the fact that no change occurs in the serum after contact with non-specific substrates.

Evidence that the interaction between specific substrate and serum may be of the nature of a specific adsorption follows from a consideration of Procedure II. The substrate designated as Typhoid "a," which has been in contact with a typhoid serum in a specific union, now so acts upon all the sera—typhoid, paratyphoid A, Staphylococcus aureus, and normal—that each serum upon subsequent dialysis gives a positive reaction (Sacs 1, 5, 9, and 13). From this we must conclude that Substrate Typhoid "a," through contact with its specific serum in Procedure I, has acquired a property which it did not inherently possess; namely, the property of causing a positive ninhydrin reaction with any serum. That this change is to be referred only to the specific action of the serum on the substrate is proved by the fact that no similar change occurs in paratyphoid A or Staphylococcus aureus substrates. In fact, these substrates, which have been treated with a non-specific serum (typhoid), have remained unchanged in the property of failing to produce a positive reaction in any serum except their homologous sera (Paratyphoid A, 2, 6, 10, 14; and Staphylococcus aureus 3, 7, 11, 15).

Note should be made of the fact that the previous contact of a substrate with a non-specific serum in no way impairs its activity when subsequently combined with a specific serum (Sacs 6 and 11).

Table 3 summarizes the results obtained with the entire series. It will be noted in Table 3 that each serum presents results analogous to those given in detail in Tables 1 and 2. It is evident that the sera of animals which have received the same immunizing treatment—15 and 16 with typhoid, 17 and 18 with paratyphoid A, 19 and 20 with Staphylococcus aureus—and also the two normal sera react in an

identical manner. Whatever the initial combination of serum and substrate, as long as the union is specific, a sensitization of substrate occurs. Whenever the primary contact occurs between a substrate and heterologous or normal sera, such a sensitization invariably fails to become manifest.

TABLE 3

SUMMARY OF RESULTS IN PROCEDURES I AND II

Procedure I		Ninhydrin Test	Procedure II								
Serum	Substrate*		Substrate†	Serum							
				15	17	19	21	16	18	20	22
15	Typhoid.....	+	Typhoid a.....	+	+	+	+				
15	Paratyphoid A..	—	Paratyphoid A b	—	+	—	—				
15	S. aureus.....	—	S. aureus c.....	—	—	+	—				
15	None.....	—	None.....	—	—	—	—				
16	Typhoid.....	+	Typhoid a.....	..	..	..	..	+	+	+	+
16	Paratyphoid A..	—	Paratyphoid A b	..	..	..	..	—	+	—	—
16	S. aureus.....	—	S. aureus c.....	..	..	..	..	—	—	+	—
16	None.....	—	None.....	..	..	..	..	—	—	—	—
17	Typhoid.....	—	Typhoid a.....	+	—	—	+‡				
17	Paratyphoid A..	+	Paratyphoid A b	+	+	+	+				
17	S. aureus.....	—	S. aureus c.....	—	—	+	—				
17	None.....	—	None.....	—	—	—	—				
18	Typhoid.....	—	Typhoid a.....	..	..	..	..	+	—	—	—
18	Paratyphoid A..	+	Paratyphoid A b	..	..	..	..	+	+	+	+
18	S. aureus.....	—	S. aureus c.....	..	..	..	..	—	—	+	—
18	None.....	—	None.....	..	..	..	..	—	—	—	—
19	Typhoid.....	—	Typhoid a.....	+	—	—	—				
19	Paratyphoid A..	—	Paratyphoid A b	—	+	—	—				
19	S. aureus.....	+	S. aureus c.....	+	+	+	+				
19	None.....	—	None.....	—	—	—	—				
20	Typhoid.....	—	Typhoid a.....	..	..	..	..	+	—	—	—
20	Paratyphoid A..	—	Paratyphoid A b	..	..	..	..	—	+	—	—
20	S. aureus.....	+	S. aureus c.....	..	..	..	..	+	+	+	+
20	None.....	—	None.....	..	..	..	..	—	—	—	—
21	Typhoid.....	—	Typhoid a.....	+	—	—	—				
21	Paratyphoid A..	—	Paratyphoid A b	—	+	—	—				
21	S. aureus.....	—	S. aureus c.....	—	—	+	—				
21	None.....	—	None.....	—	—	—	—				
22	Typhoid.....	—	Typhoid a.....	..	..	..	..	+	—	—	—
22	Paratyphoid A..	—	Paratyphoid A b	..	..	..	..	—	+	—	—
22	S. aureus.....	—	S. aureus c.....	..	..	..	..	—	—	+	—
22	None.....	—	None.....	..	..	..	..	—	—	—	—

* Sera and substrates in contact on ice 16 hr. Substrates separated by centrifugation and washed twice with salt solution. Sera dialyzed 16 hr.

† Substrates from Procedure I placed in contact with fresh sera on ice 16 hr. Centrifugated cold. Sera dialyzed 16 hr.

‡ An aberrant positive reaction for which no explanation can be given.

SUMMARY

The present work, confirming the studies of Bronfenbrenner, demonstrates that the Abderhalden reaction may be divided into 2 phases. The first phase involves a sensitization of substrate by its

specific serum. The second phase represents an autodigestion of the serum, which is not due to specific causes.

In the sensitization of substrate an absolute specificity obtains. Whether or not this sensitization is of the nature of an antigen-antibody reaction forms at present the subject of a further investigation.